

Attorney Docket No.: **PTQ-0038**
Inventors: **Van Eyk and Arrell**
Serial No.: **09/942,498**
Filing Date: **August 30, 2001**
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This listing of the claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

Claim 1: (currently amended) A method for identifying an agent which inhibits damage to cardiac and skeletal muscle comprising: ~~assessing the ability of a potential muscle protective agent to increase myosin light chain 1 (MLC1) phosphorylation~~

(a) combining adenosine triphosphate and a test agent with purified myosin, myosin light chain 1 (MLC1) or isoform thereof, or myofilament or skinned muscle fiber in the presence of an enzyme that modulates phosphorylation of MLC1; and

(b) monitoring phosphorylation of MLC1 in the purified myosin, myosin light chain 1 (MLC1) or isoform thereof, or myofilament or skinned muscle fiber in the presence and absence of the test agent, wherein an increase in MLC1 phosphorylation in the purified myosin, myosin light chain 1 (MLC1) or isoform thereof, or myofilament or skinned muscle fiber in the presence of the test agent is indicative of the test agent inhibiting damage to cardiac and skeletal muscle.

Claim 2: (canceled)

Claim 3: (currently amended) ~~The method of claim 1 wherein the~~

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~~ability of the potential muscle protective agent to increase MLC1 phosphorylation is assessed in A method for identifying an agent which inhibits damage to cardiac and skeletal muscle comprising:~~

- (a) ~~combining a test agent with isolated myocytes or whole hearts either isolated using Langendorff apparatus or in vivo; and~~
- (b) ~~monitoring phosphorylation of MLC1 in the isolated myocytes or whole hearts in the presence and absence of the test agent, wherein an increase in MLC1 phosphorylation in the isolated myocytes or whole hearts in the presence of the test agent is indicative of the test agent inhibiting damage to cardiac and skeletal muscle.~~

Claims 4-11: (previously canceled)

Claim 12: (currently amended) A method for identifying new therapeutic targets for agents which inhibit damage to cardiac and skeletal muscle comprising: ~~identifying kinases or phosphatases that modulate MLC1 phosphorylation status~~

- (a) ~~combining an enzyme with purified myosin, myosin light chain 1 (MLC1) or isoform thereof, or myofilament or skinned muscle fiber in the presence of adenosine triphosphate; and~~
- (b) ~~monitoring phosphorylation of MLC1 in the purified myosin, myosin light chain 1 (MLC1) or isoform thereof, or myofilament or skinned muscle fiber in the presence and absence of the enzyme,~~

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wherein modulation of MLC1 phosphorylation status ~~by the identified kinase or phosphatase in the presence of the enzyme~~ is indicative of the ~~identified kinase or phosphatase enzyme~~ being a therapeutic target for an agent which inhibits damage to cardiac or skeletal muscle.

Claim 13: (previously canceled)

Claim 14: (currently amended) The method of ~~claim 2 claim 1~~ wherein the myosin, myosin light chain 1, or ~~isoforms isoform~~ thereof are obtained from a biological sample using IN Sequence extraction.

Claim 15: (previously canceled)

Claim 16: (currently amended) The method of claim 1 wherein MLC1 phosphorylation is increased by ~~modulation decreasing activity~~ of a phosphatase that acts on MLC1 phosphorylation.

Claim 17: (currently amended) The method of claim 1 wherein MLC1 phosphorylation is increased by ~~modulation increasing activity~~ of a kinase that acts on MLC1 phosphorylation.

Claim 18: (currently amended) The method of claim 1 wherein the increased MLC1 phosphorylation occurs at one or more of residues Thr64, Ser194 ~~or and~~ Ser195 of human MLC1.

Claim 19: (currently amended) The method of claim 1 wherein the increased MLC1 phosphorylation occurs at one or more of residues Thr69 ~~or and~~ Ser200 of rat MLC1.

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Claim 20: (currently amended) The method of claim 1 wherein the ~~muscle protective identified~~ agent ~~identified~~ protects against muscle damage caused by ~~cardiomyopathies~~ a cardiomyopathy, hypertension, or a free ~~radicals~~ radical.

Claim 21: (currently amended) The method of claim 1 wherein the ~~muscle protective identified~~ agent ~~identified~~ protects against muscle damage caused by ischemia, hypoxia, or ischemia/hypoxia with reperfusion.

Claim 22: (previously added) The method of claim 1 wherein the ability of the agent to increase MLC1 phosphorylation is assessed *in vitro* in myofilament or skinned muscle fibers.

Claim 23: (new) The method of claim 3 wherein MLC1 phosphorylation is increased by decreasing activity of a phosphatase that acts on MLC1 phosphorylation.

Claim 24: (new) The method of claim 3 wherein MLC1 phosphorylation is increased by increasing activity of a kinase that acts on MLC1 phosphorylation.

Claim 25: (new) The method of claim 3 wherein the increased MLC1 phosphorylation occurs at one or more of residues Thr64, Ser194 and Ser195 of human MLC1.

Claim 26: (new) The method of claim 3 wherein the increased MLC1 phosphorylation occurs at one or more of residues Thr69 and Ser200

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of rat MLC1.

Claim 27: (new) The method of claim 3 wherein the identified agent protects against muscle damage caused by a cardiomyopathy, hypertension, or a free radical.

Claim 28: (new) The method of claim 3 wherein the identified agent protects against muscle damage caused by ischemia, hypoxia, or ischemia/hypoxia with reperfusion.

Claim 29: (new) The method of claim 12 wherein the myosin, myosin light chain 1, or isoform thereof are obtained from a biological sample using IN Sequence extraction.

Claim 30: (new) The method of claim 12 wherein the enzyme is a phosphatase and decreasing activity of the phosphatase increases MLC1 phosphorylation.

Claim 31: (new) The method of claim 12 wherein the enzyme is a kinase and increasing activity of the kinase increases MLC1 phosphorylation.

Claim 32: (new) The method of claim 12 wherein MLC1 phosphorylation is increased at one or more of residues Thr64, Ser194 and Ser195 of human MLC1.

Claim 33: (new) The method of claim 12 wherein MLC1 phosphorylation is increased at one or more of residues Thr69 and Ser210 of rat MLC1.